Articular cartilage changes after immobilization in a rat knee contracture model

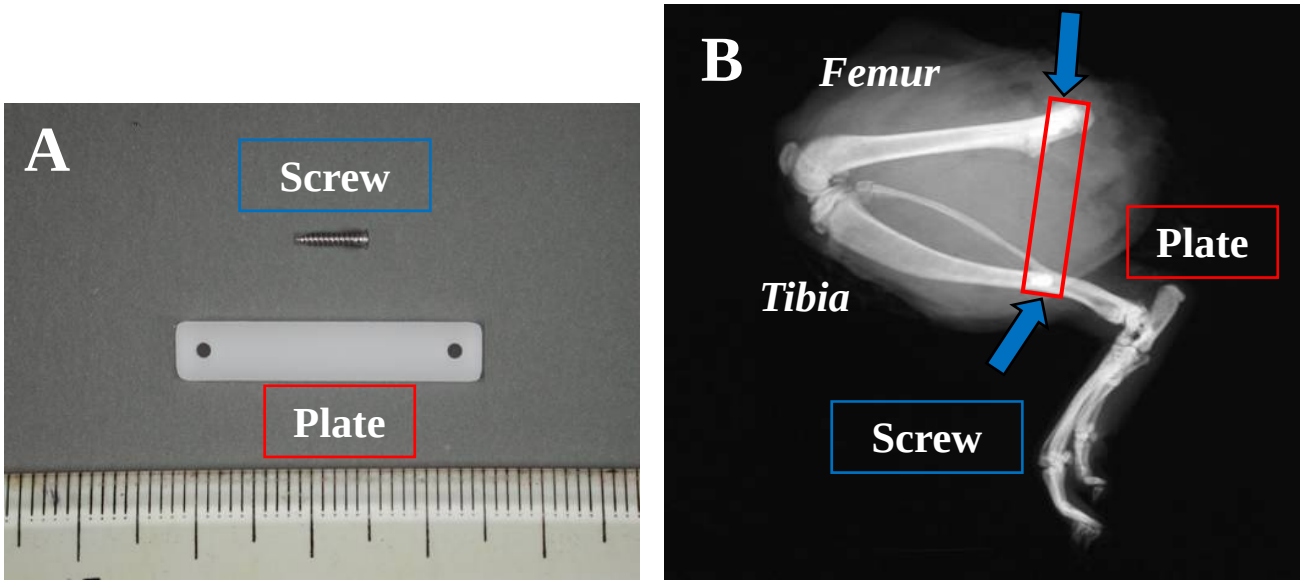


Figure 1. Methods of immobilization

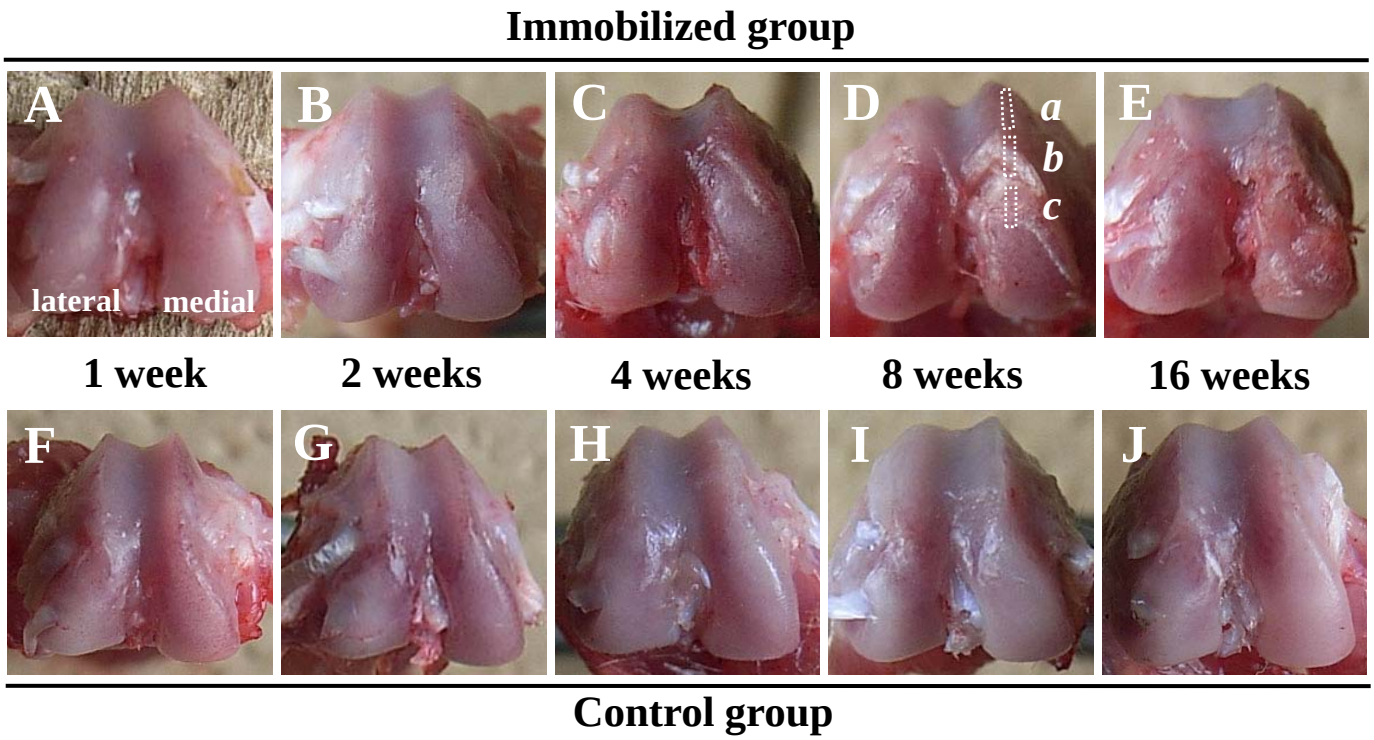


Figure 2. Gross observation

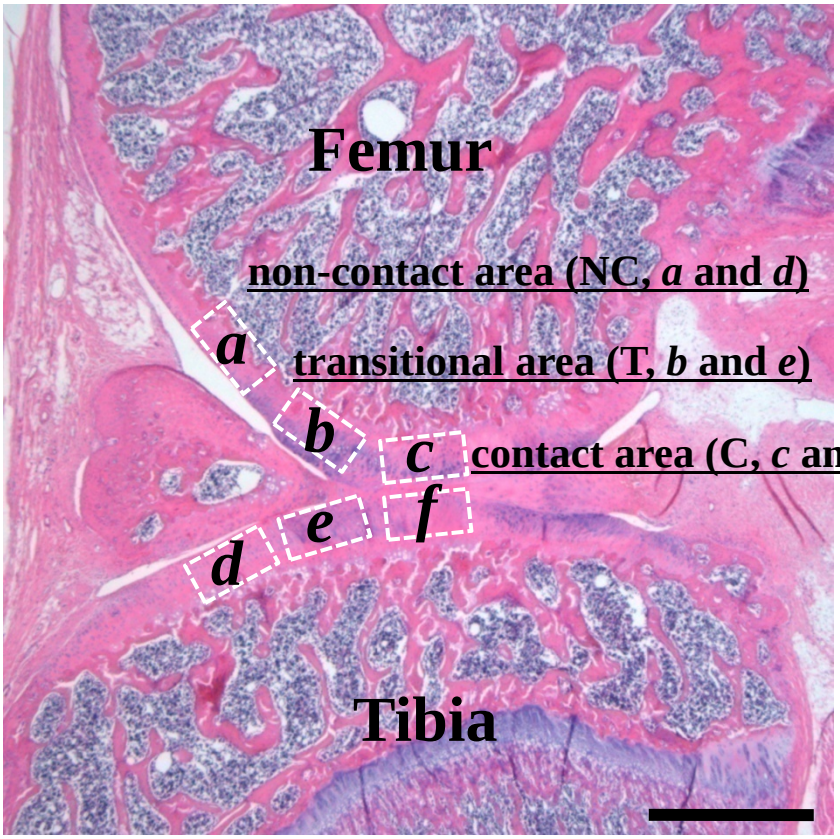


Figure 3. Evaluated areas

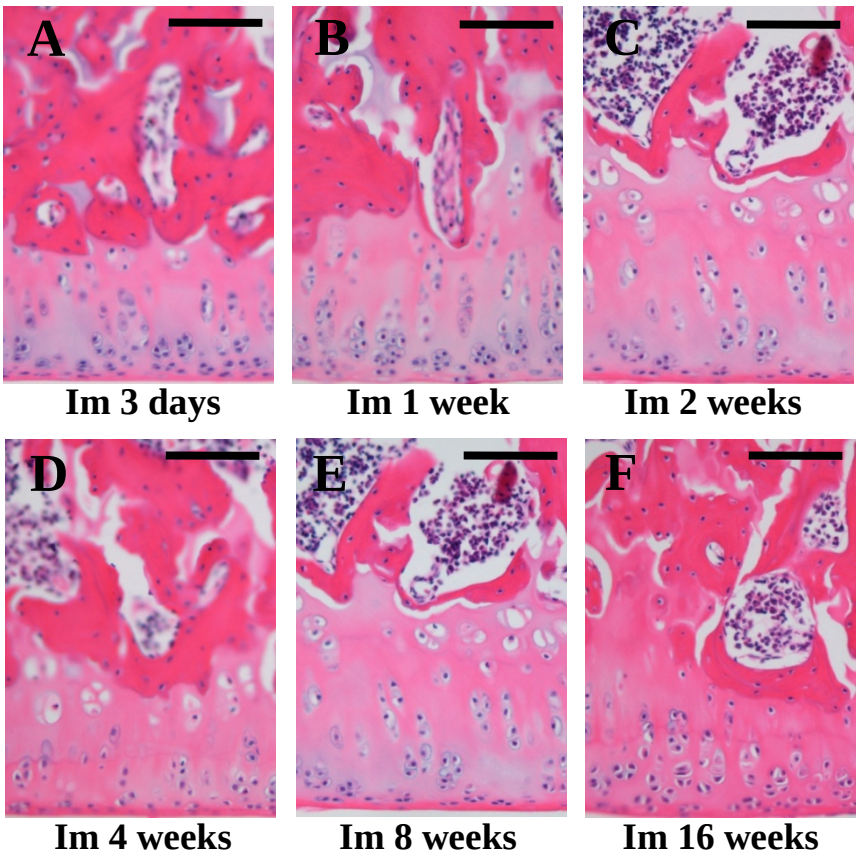


Figure 4. H-E staining in the non-contact area (*a*)

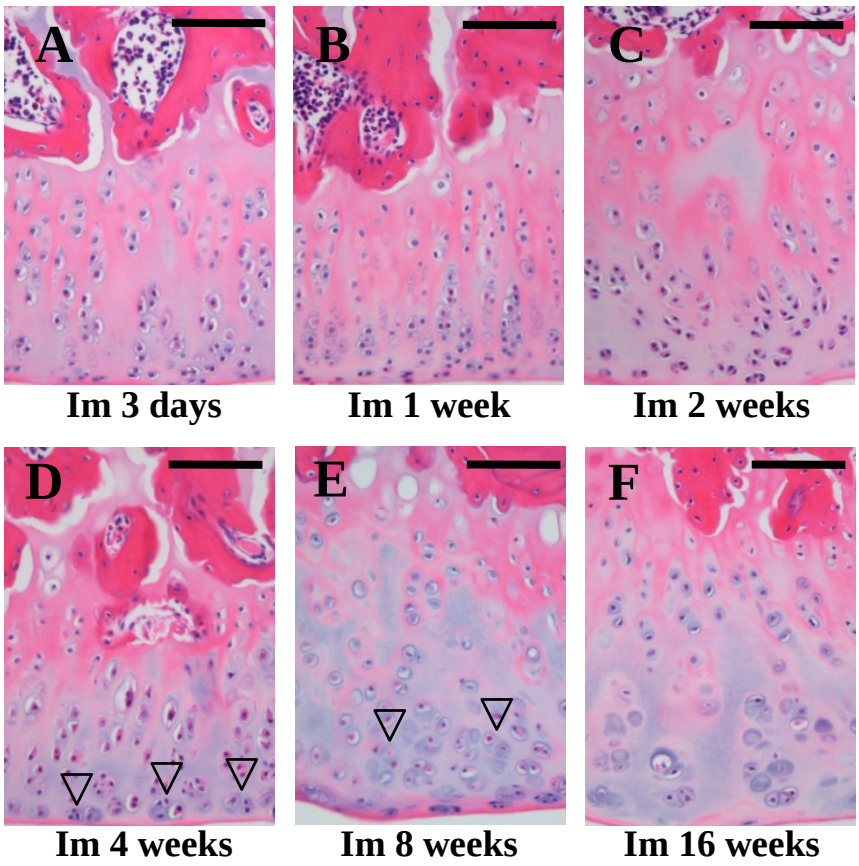


Figure 5. H-E staining in the transitional area (*b*)

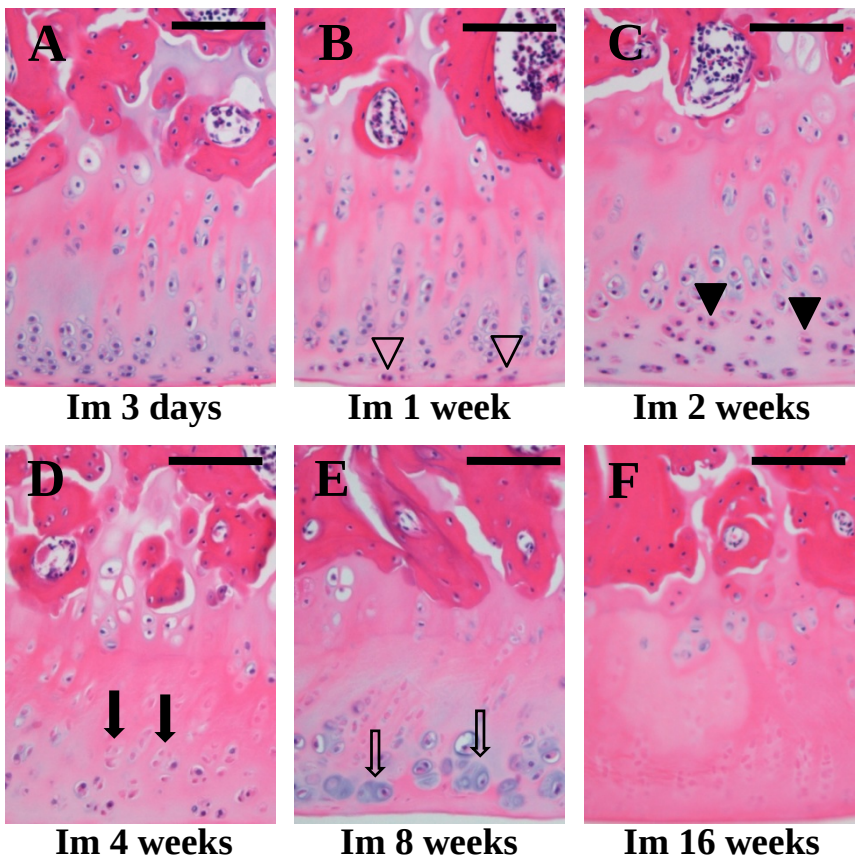


Figure 6. H-E staining in the contact area (*c*)

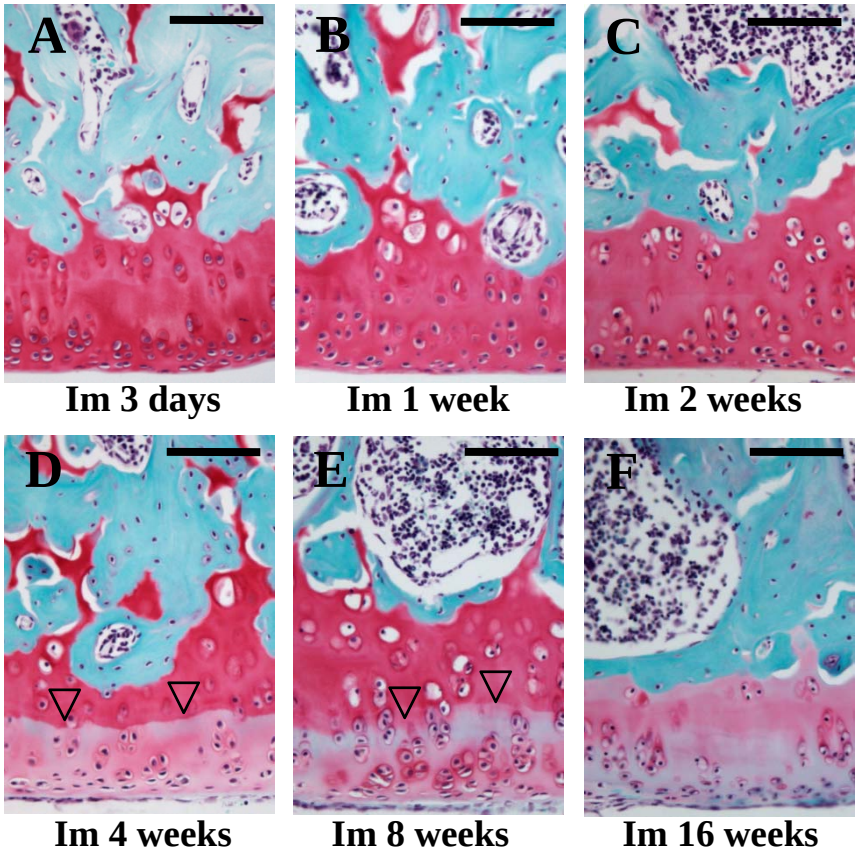


Figure 7. S-O staining in the non-contact area (*a*)

Transitional area (*b*)

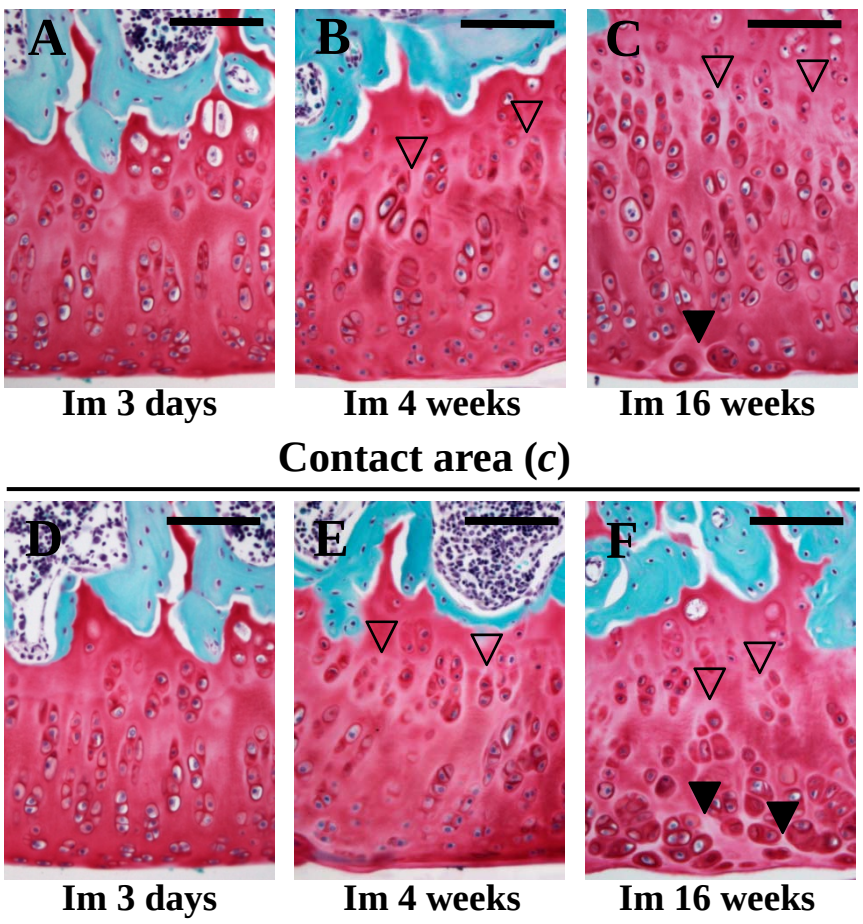


Figure 8. S-O staining in the transitional and contact area

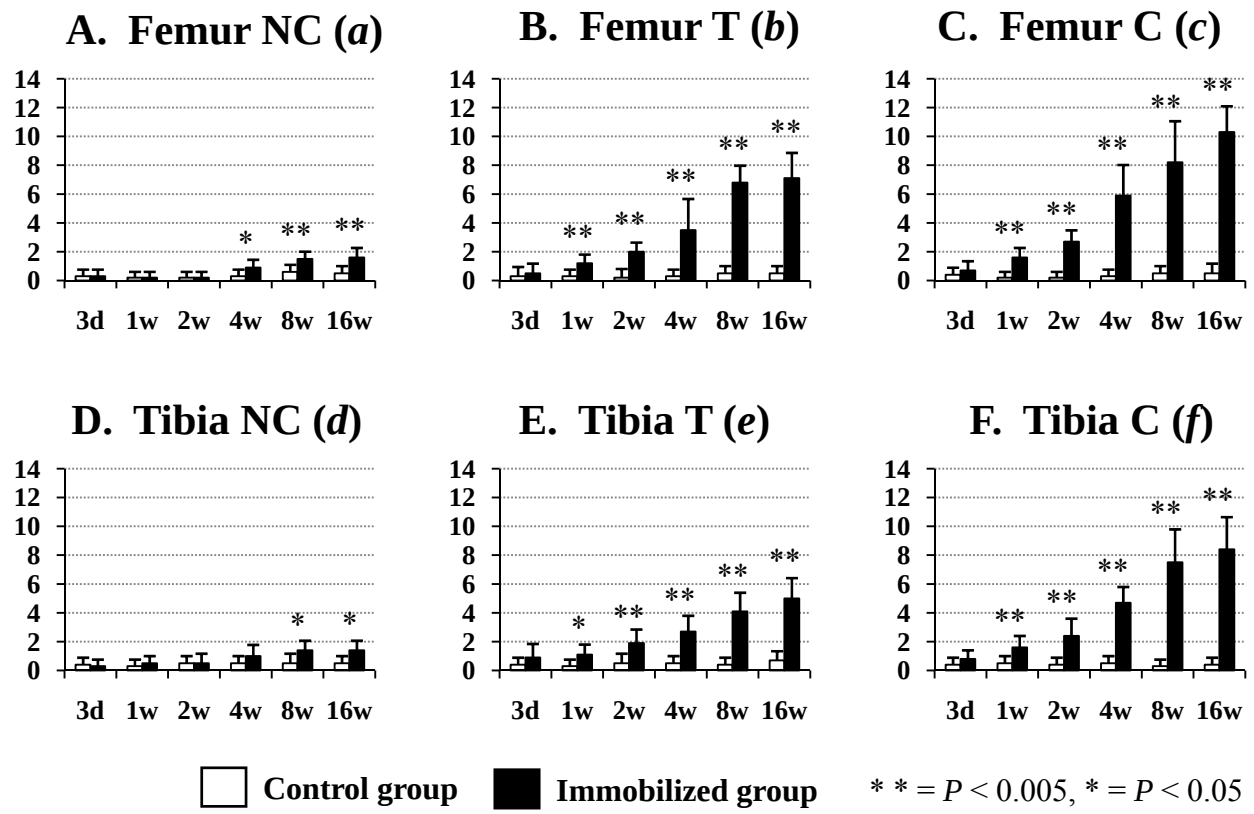


Figure 9. Modified Mankin's score

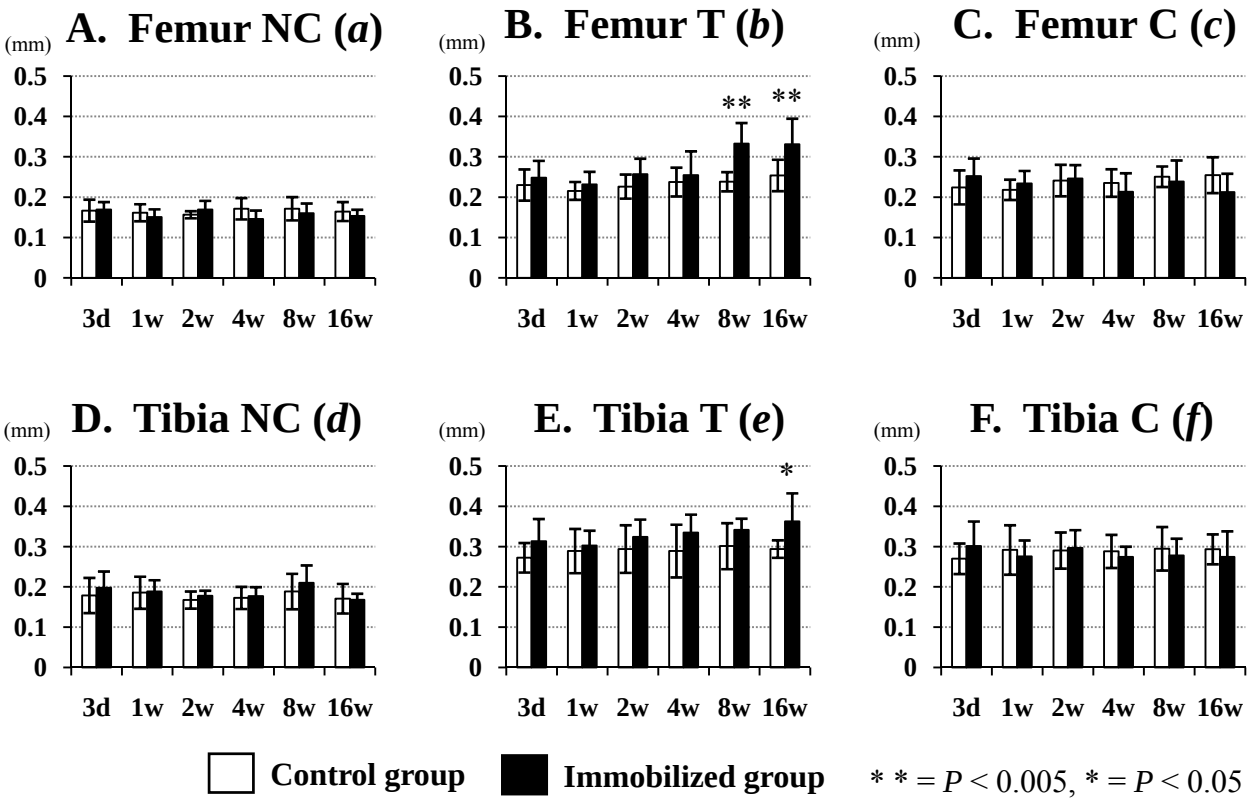


Figure 10. Articular cartilage thickness

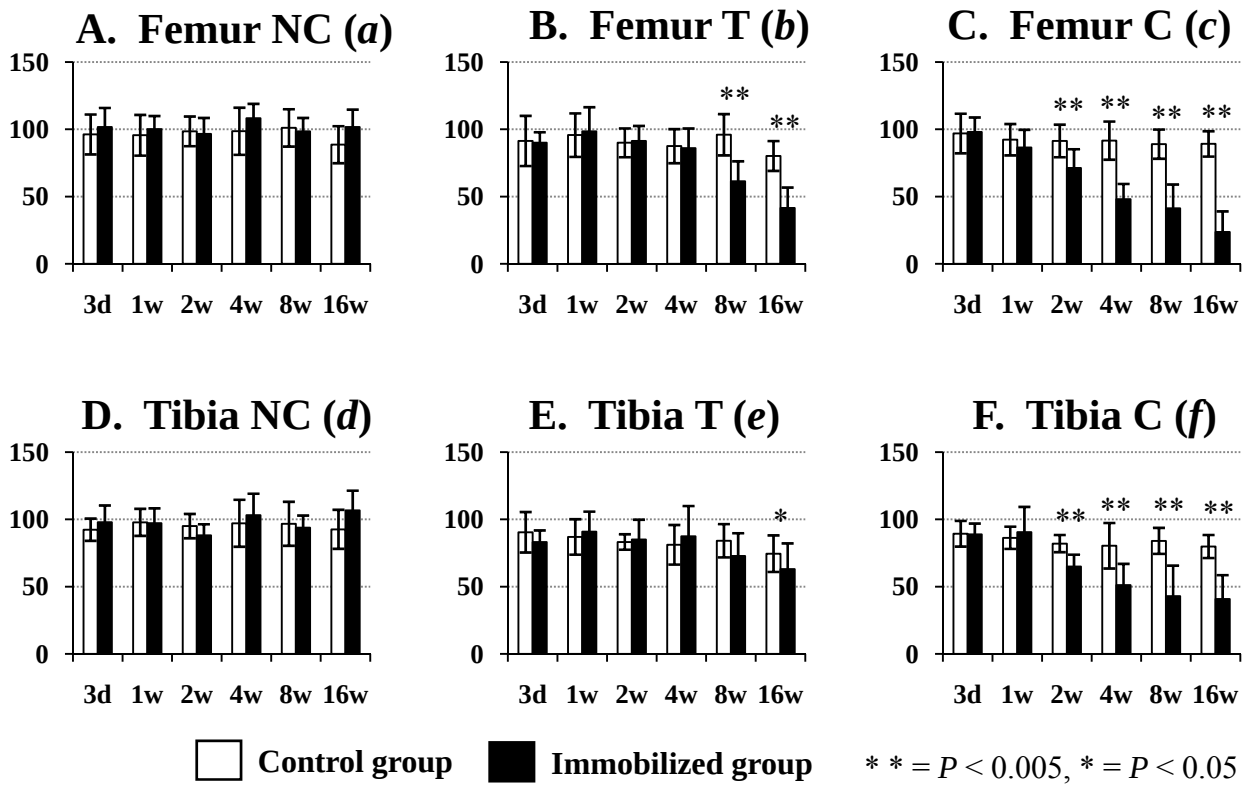


Figure 11. Number of chondrocytes

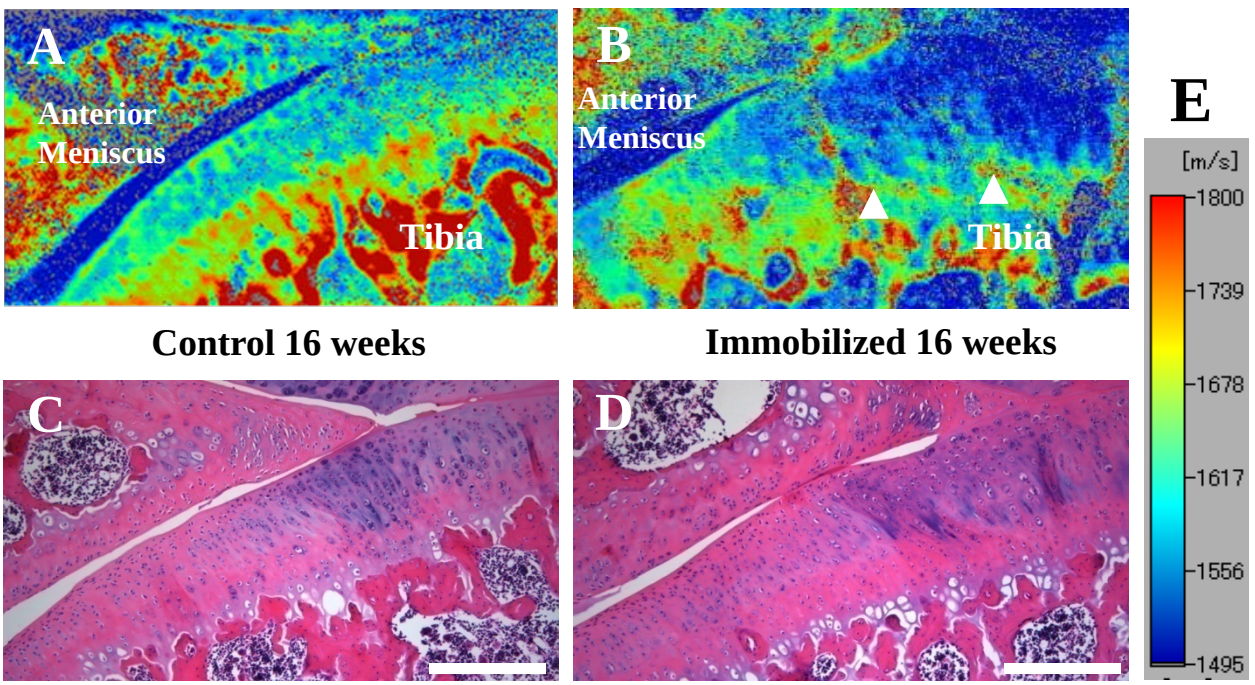


Figure 12. SAM (Scanning acoustic microscopy)

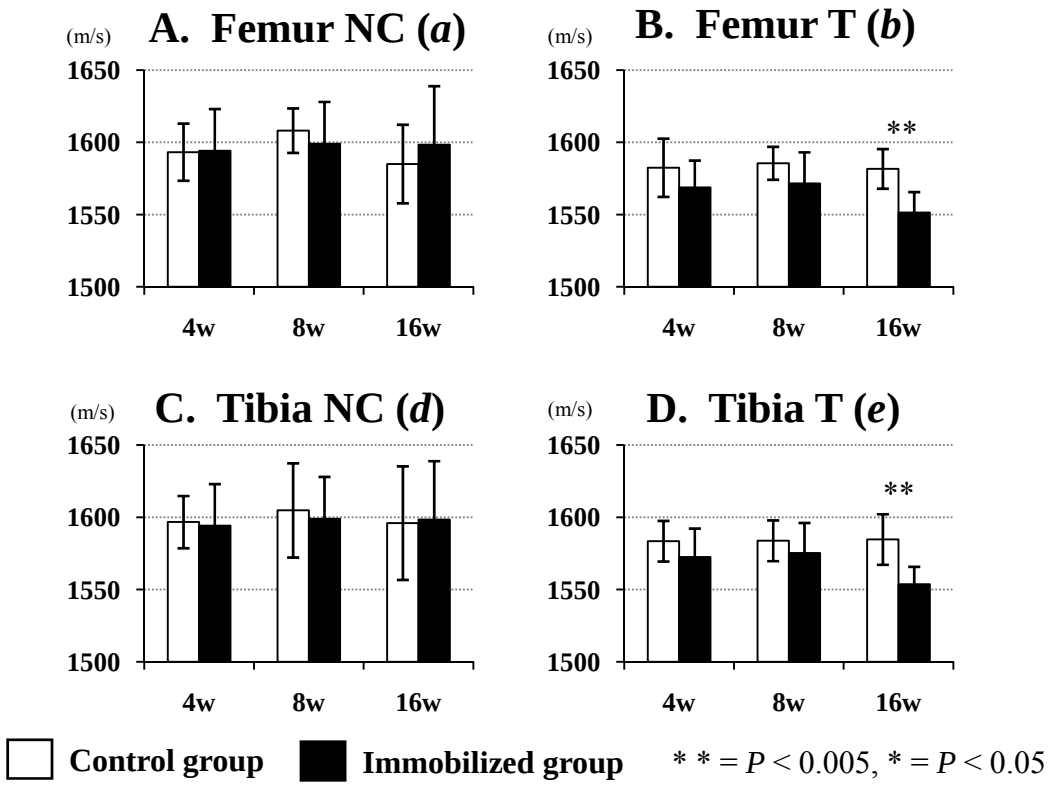


Figure 13. Sound speed of articular cartilage

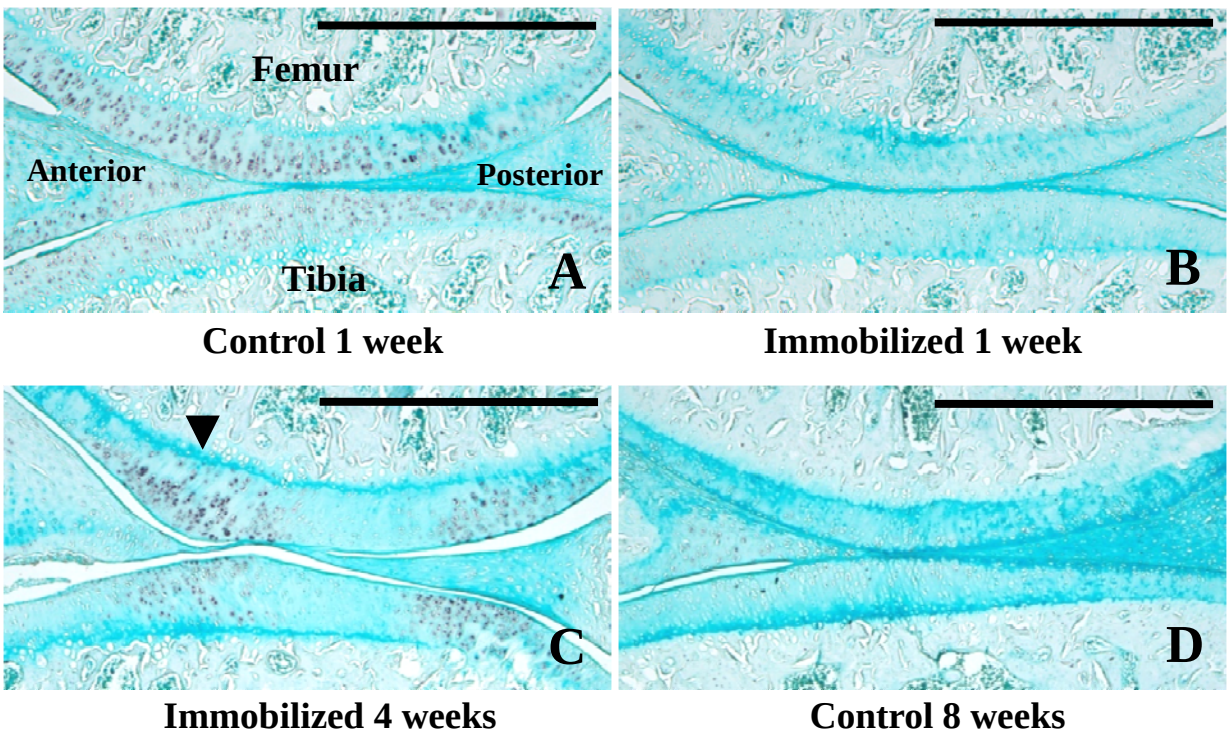


Figure 14. ISH of type II collagen

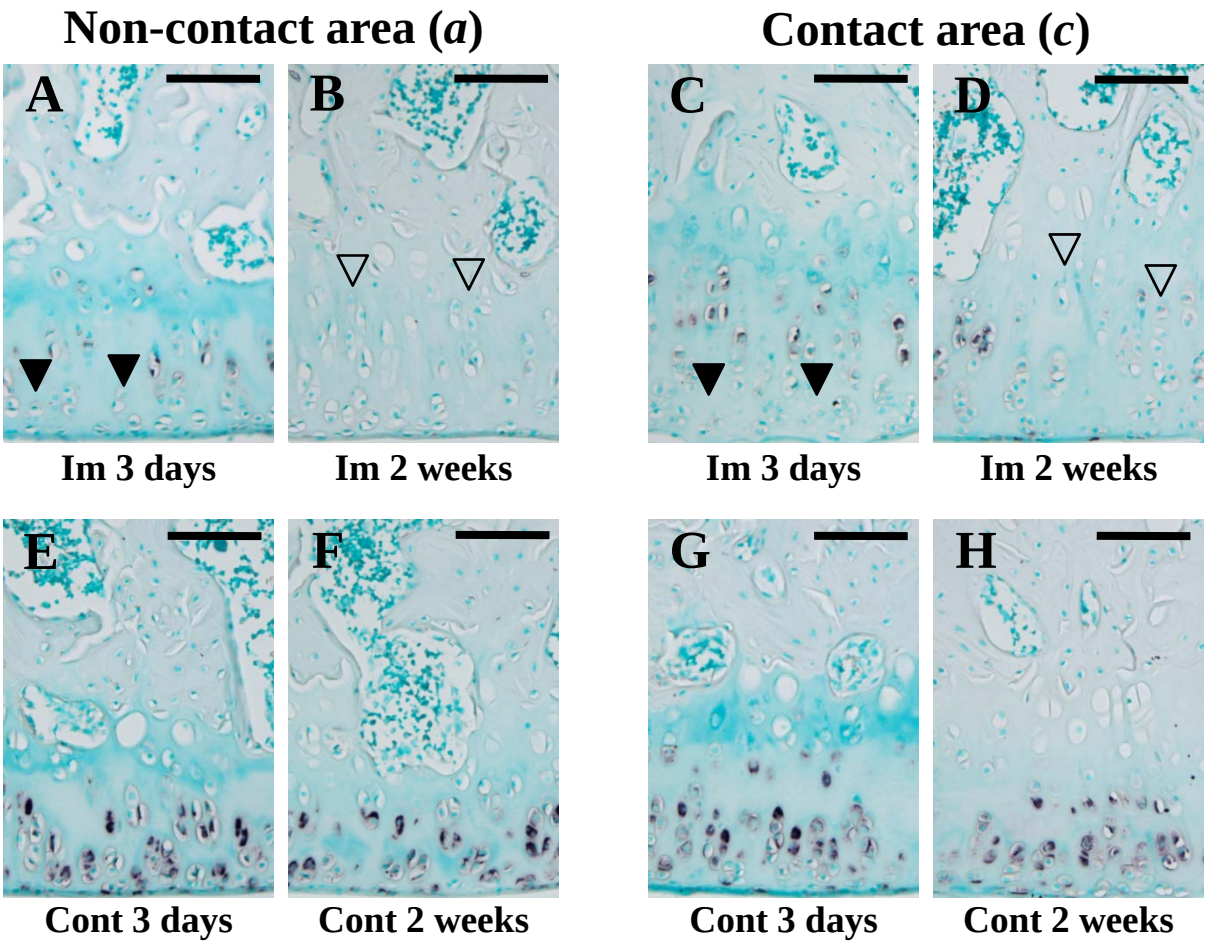


Figure 15. ISH of type II collagen (non-contact & contact area)

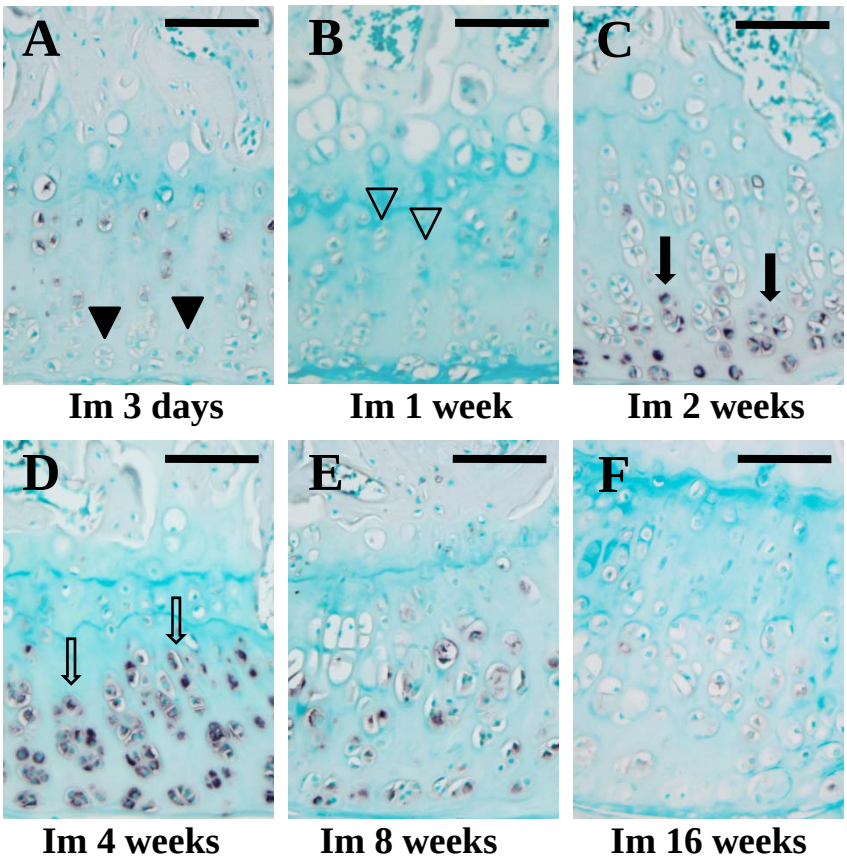


Figure 16. ISH of type II collagen (transitional area, *b*)

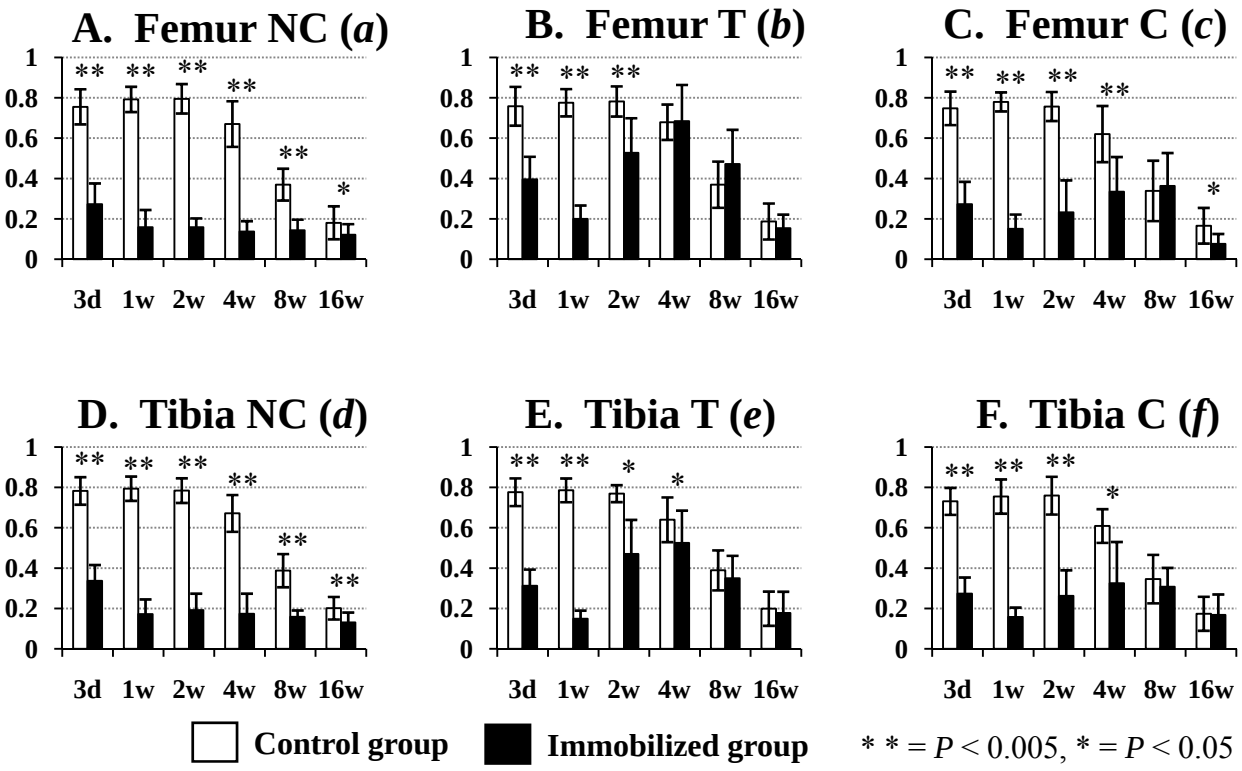


Figure 17. Positive cell ratio of type II collagen

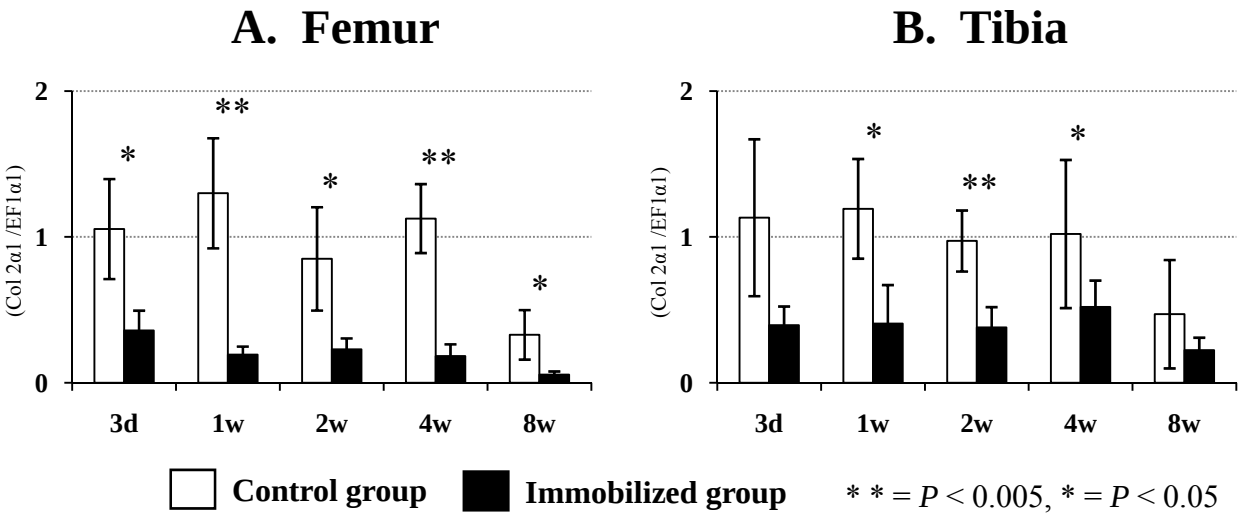


Figure 18. qPCR of type II collagen

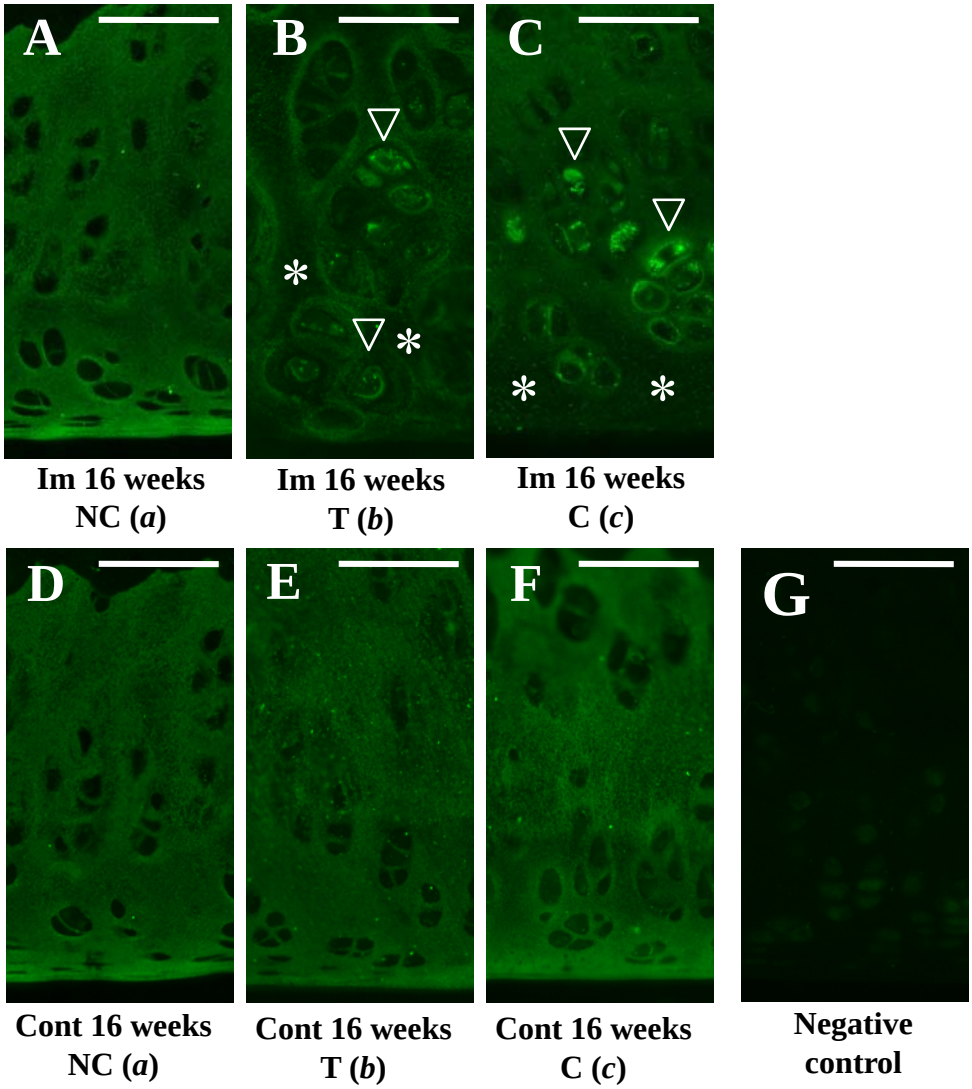


Figure 19. IHC of type II collagen

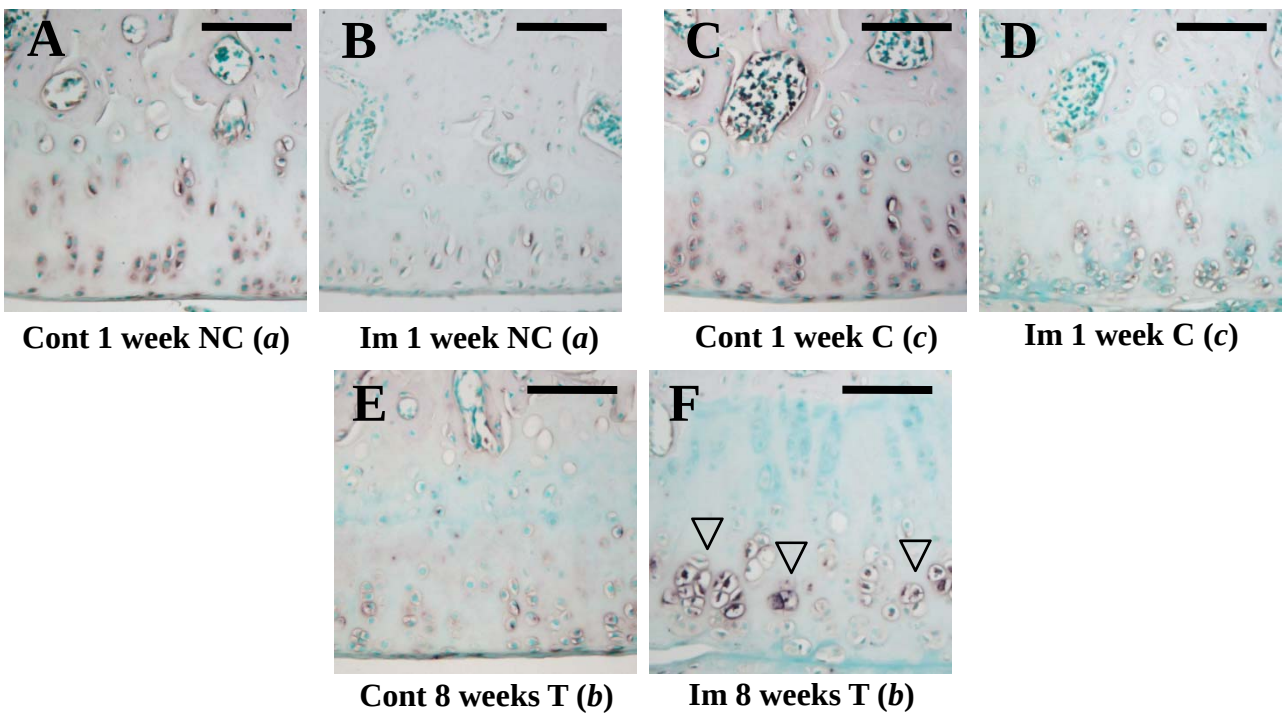
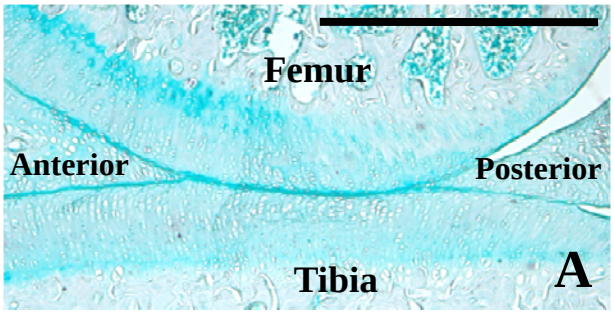
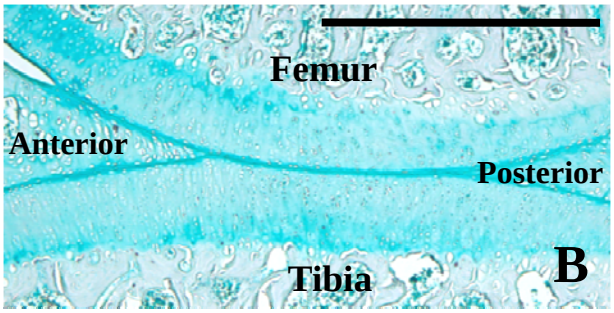


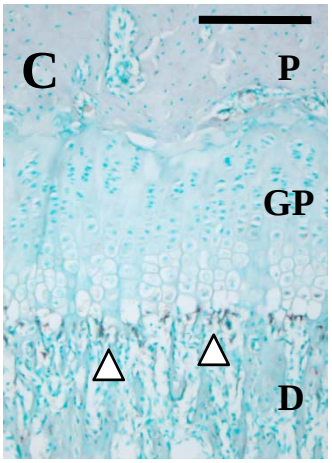
Figure 20. ISH of MMP-8



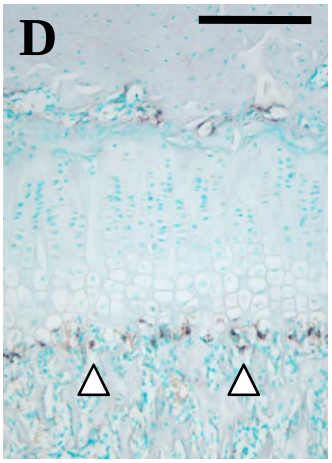
Control 1 week



Immobilized 1 week



Control 1 week



Immobilized 1 week

Figure 21. ISH of MMP-13

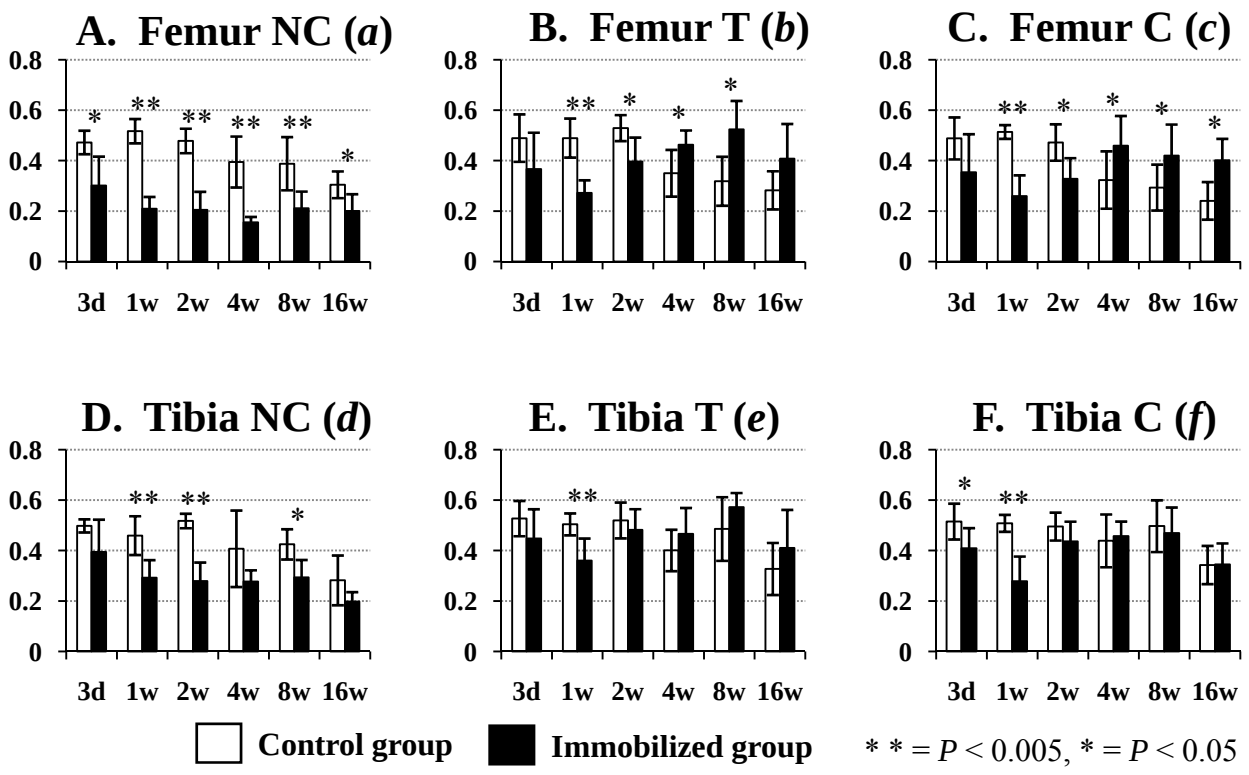


Figure 22. Positive cell ratio of MMP-8

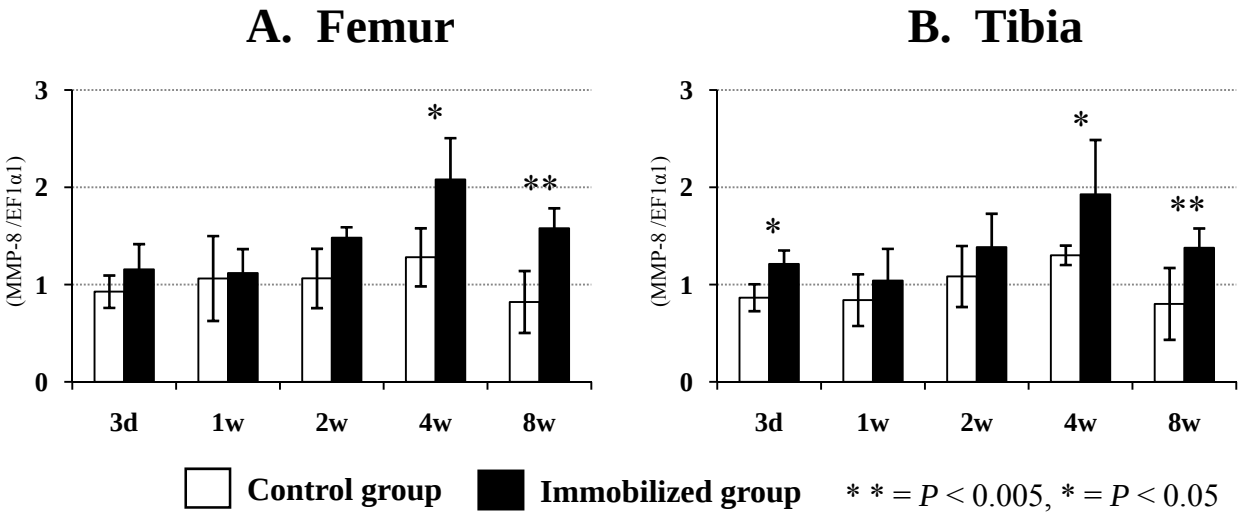


Figure 23. qPCR of MMP-8

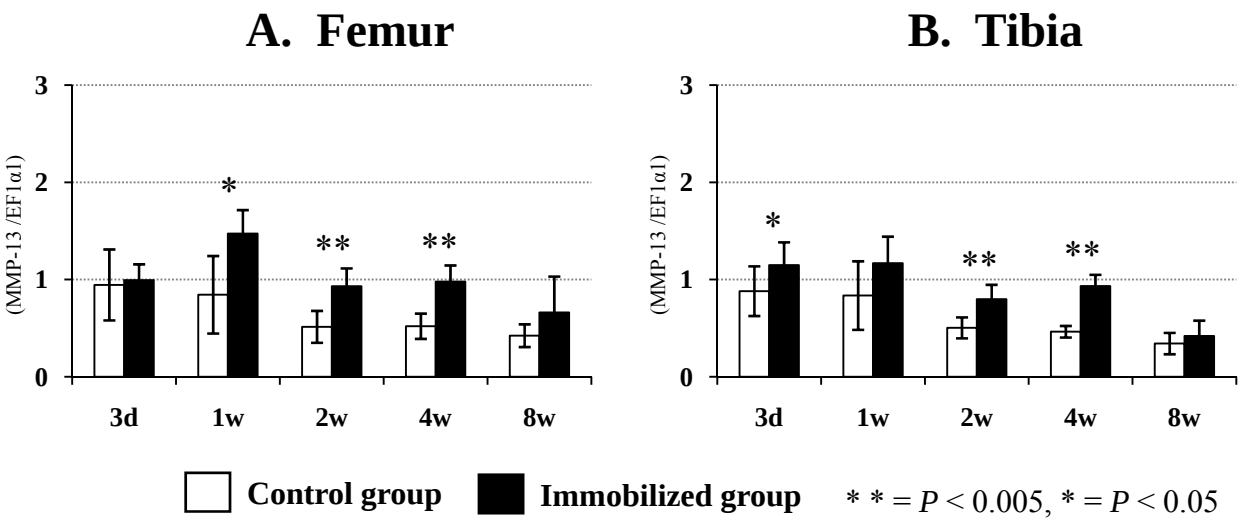


Figure 24. qPCR of MMP-13

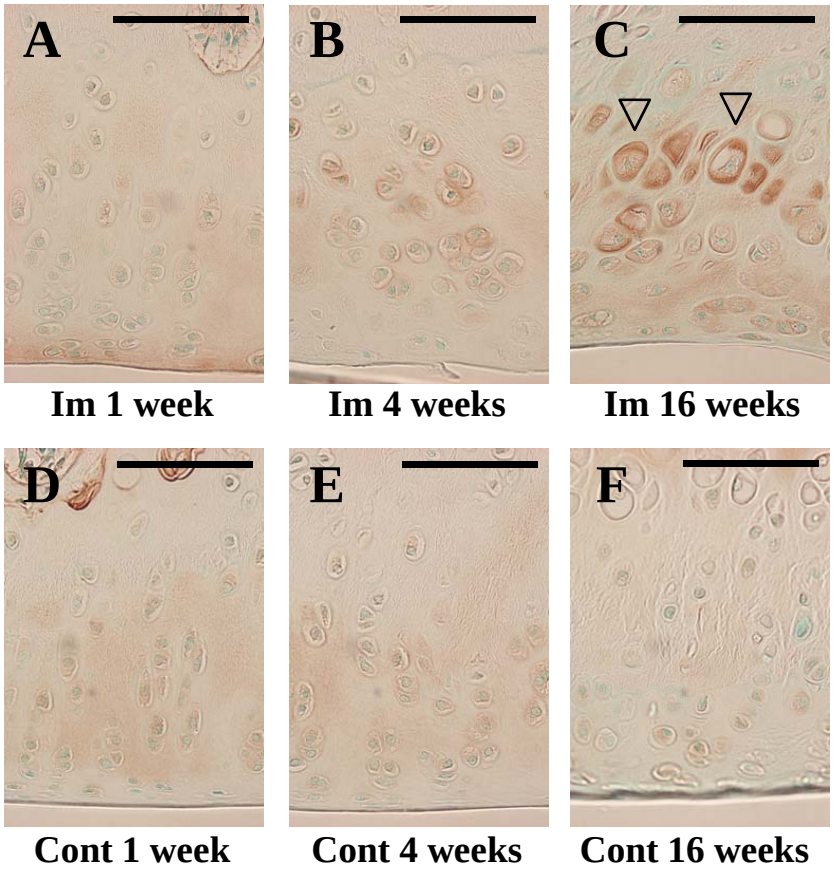


Figure 25. IHC of MMP-13 (*c*)

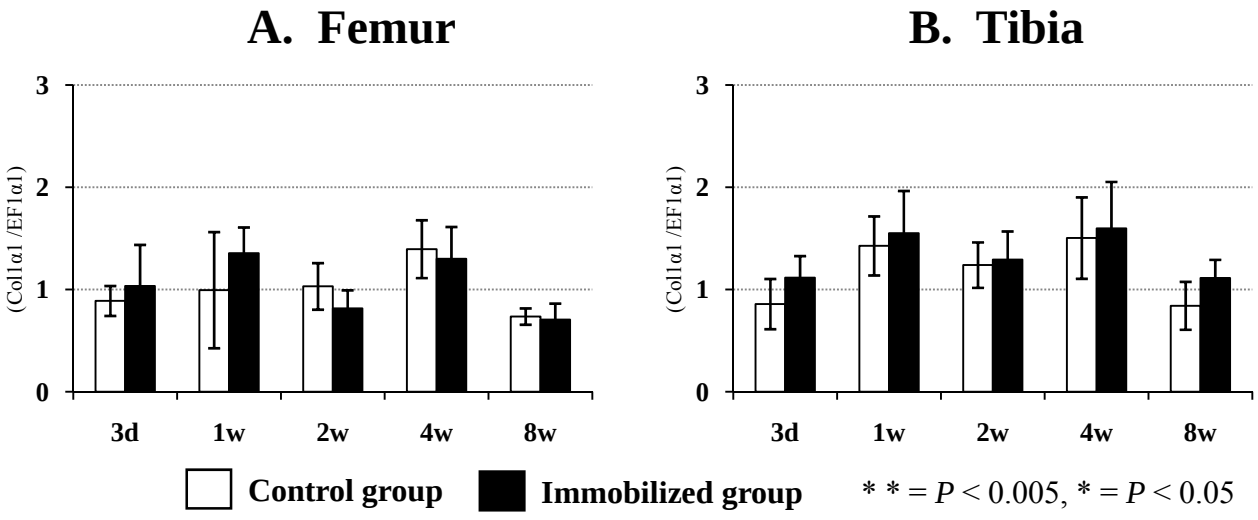
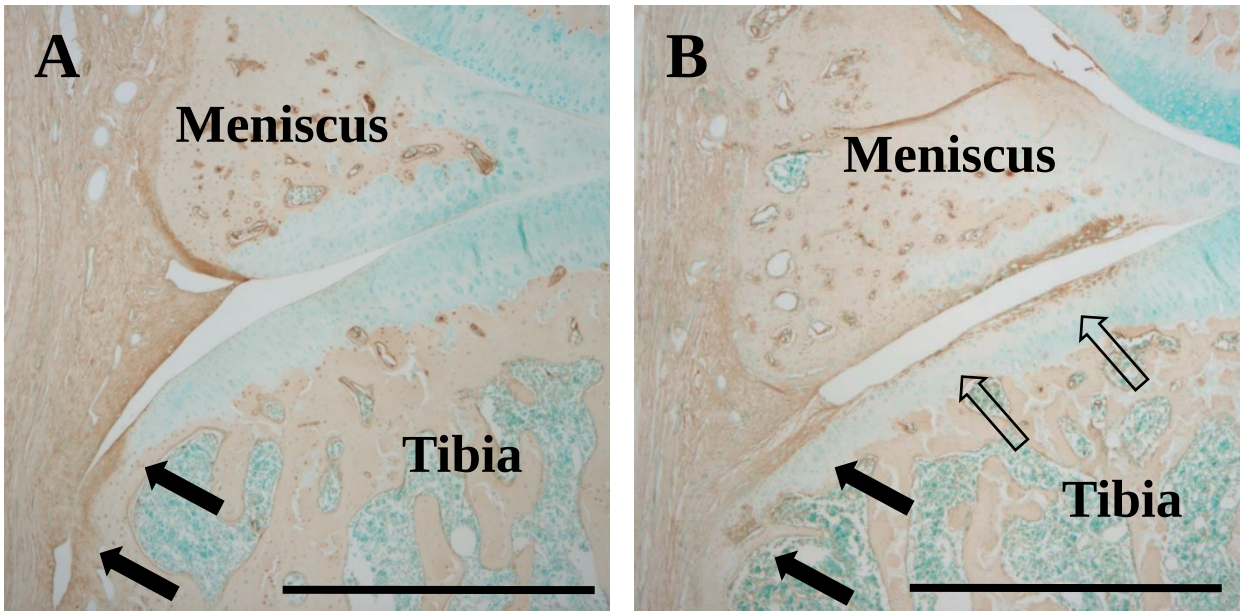


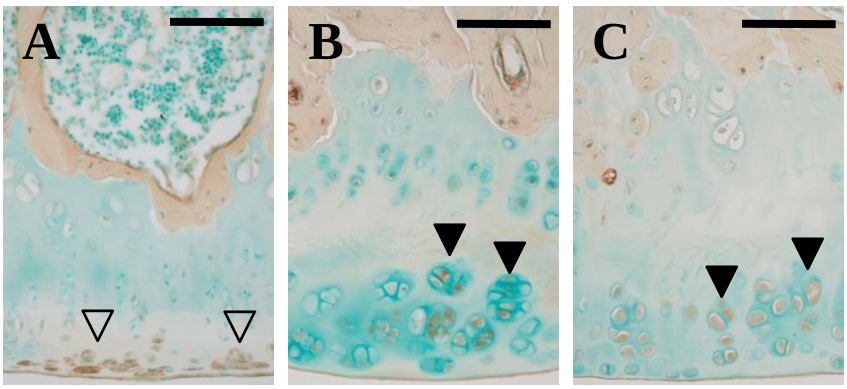
Figure 26. qPCR of type I collagen



Control 4 weeks

Immobilized 4 weeks

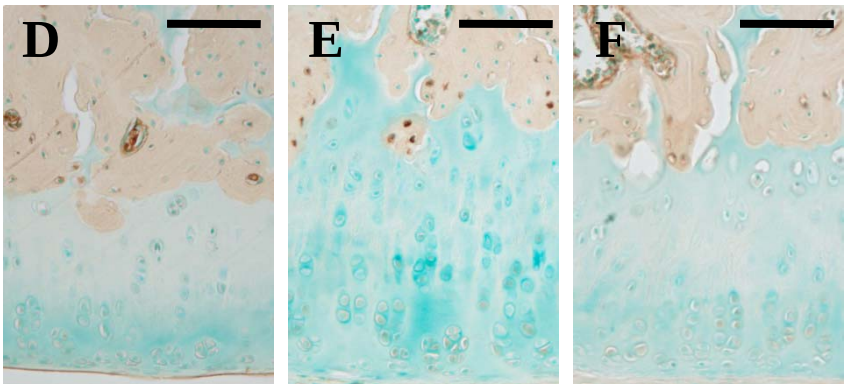
Figure 27. IHC of type I collagen



Im 8 weeks
NC (*a*)

Im 8 weeks
T (*b*)

Im 8 weeks
C (*c*)



Cont 8 weeks
NC (*a*)

Cont 8 weeks
T (*b*)

Cont 8 weeks
C (*c*)

Figure 28. IHC of type I collagen

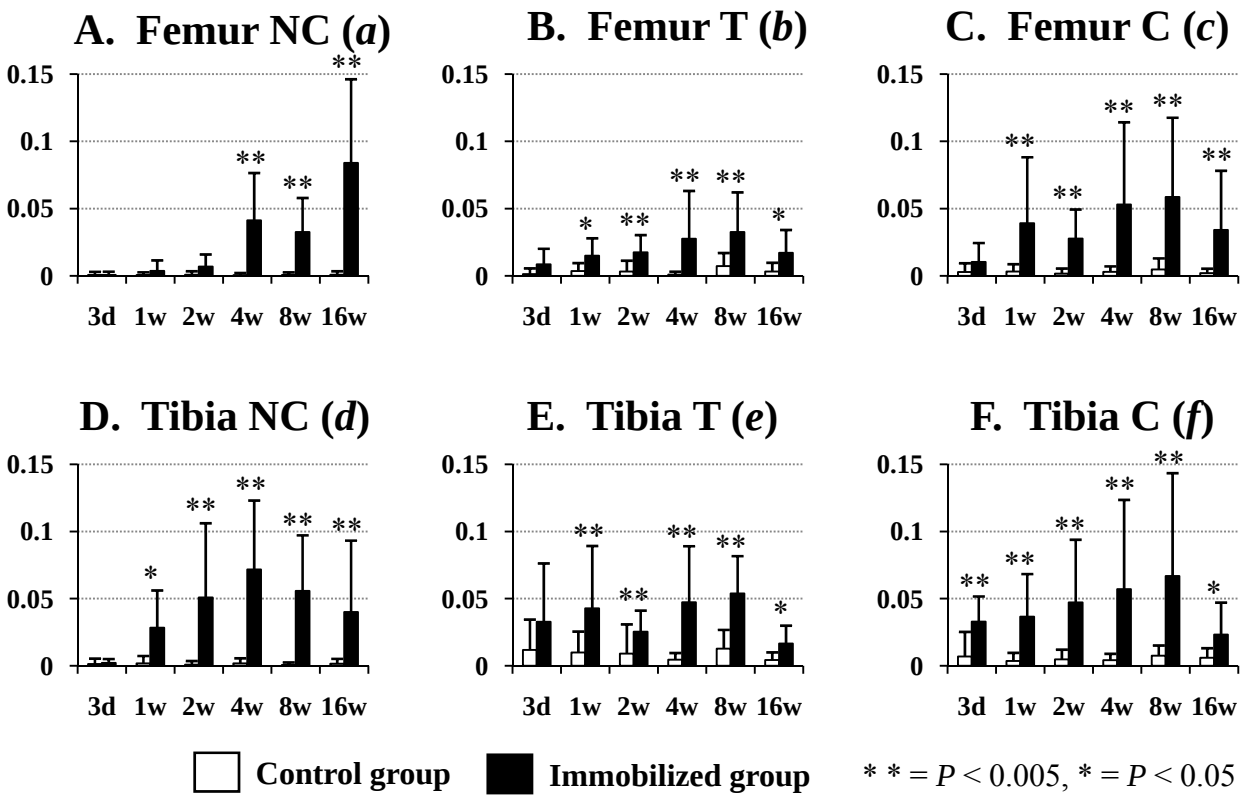


Figure 29. Positive cell ratio of type I collagen

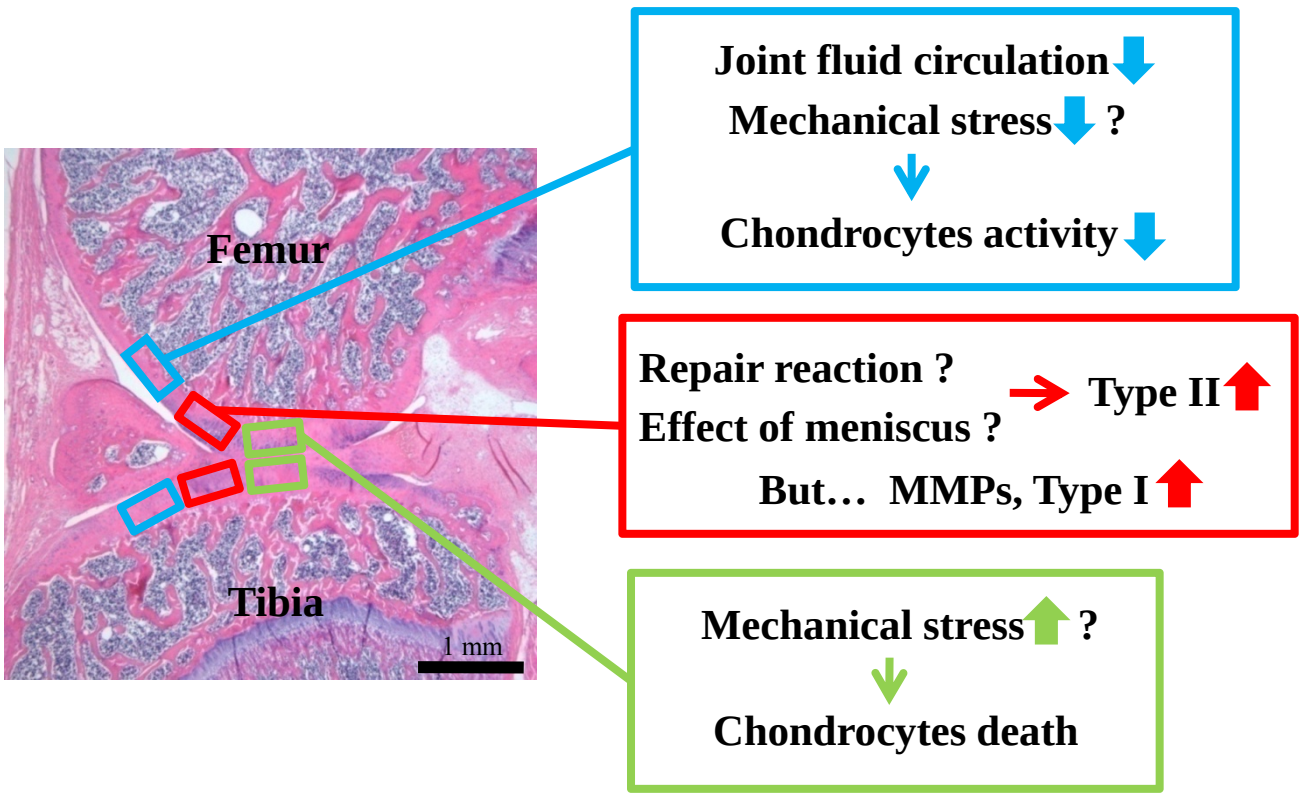


Figure 30. Changes of articular cartilage after rigid immobilization

Table 1. Histological grading scheme (Modified Mankin's score)

	Grade
I. Structure	
Normal	0
Slight surface irregularities	1
Moderate surface irregularities	2
Severe surface irregularities	3
Cleft in transitional zone	4
Cleft in radial zone	5
Cleft in calcified zone	6
Loss of transitional zone	7
Loss of radial zone	8
Loss of calcified zone	9
Complete disorganization	10
II. Cell	
1. Tangenital zone	
Normal	0
Swelling of cells	1
Disappearance of cells	2
2. Transitional and radial zone	
Normal	0
Slight hypercellularity	1
Moderate hypercellularity	2
Severe hypercellularity	3
Slight cloning	4
Moderate cloning	5
Severe cloning	6
Slight hypocellularity	7
Moderate hypocellularity	8
Severe hypocellularity	9
Disappearance of cells	10
III. Safranin O staining	
Normal	0
Slight reduction	1
Moderate reduction	2
Severe reduction	3
No dye noted	4
IV. Tidemark	
Intact	0
Multilayered	1
Indistinct	2
Crossed by blood vessels	3
V. Pannus formation	
Normal	0
Slight	1
Moderate	2
Marked	3

Table 2. Oligonucleotide primers to prepare cDNA for RNA probes

		Primer
Mouse pro α1(II) collagen (545 bp)	upper	5’ TGAAGACATCCGCAGCCCC 3’
	lower	5’ ATAATGGGAAGGCGGGAGG 3’
Rat pro α1(I) collagen (1492 bp)	upper	5’ CCCAAGCTTGCGTGGTGTGGTCGGTCT 3’
	lower	5’ GATGGAGGGAGTTTACACGA 3’
Rat MMP-8 (1083 bp)	upper	5’ CACTATGATTGCCGAGAA 3’
	lower	5’ AACACGCTTGCTATGCTA 3’
Rat MMP-13 (865 bp)	upper	5’ TGACTATGCGTGGCTGGA 3’
	lower	5’ GGGAAGGGGCTAATGAACA 3’

Table 3. Sequences of primers for quantitative RT-PCR

		Primer
Col2α1	upper	5’ AGCAGCAAGAGCAAGGAGAAGAA 3’
	lower	5’ CAGTGGACAGTAGACGGAGGAAAGT 3’
Col1α1	upper	5’ GACGCATGGCCAAGAAGACA3’
	lower	5’ GTTTTTGGGGGTTGGGACAGT3’
MMP-8	upper	5’ TGACCCCCACCTGAGATTTGA 3’
	lower	5’ TAGGTAGGAAGGGCCAGAACAGAG 3’
MMP-13	upper	5’ TACGAGCATCCATCCCGAGACC 3’
	lower	5’ AACCGCAGCACTGAGCCTTTTC 3’
EF1α1	upper	5’ TGATGCCCCAGGACACAGAGACT 3’
	lower	5’ CCCAACACCAGCAGCAACAATC 3’

Table 4. Summary of the results

1. Joint immobilization induces cartilage degeneration
2. The changes in each area are different :

   Non-contact area : Loss of Proteoglycan between tidemark and cartilage surface
   Transitional area : Hypertrophic changes
   Increase of cartilage thickness
   Contact area : Atrophic changes
   Loss of chondrocytes
3. Sound speed of articular cartilage decreases after joint immobilization
4. Expression of Type II collagen decreases after joint immobilization
5. Hypertrophic chondrocytes express type II collagen
6. Immunostaining of type II collagen in the non-contact area is maintained
   The staining in the transitional and contact areas is decreased
7. Expression of MMP-8 decreases after joint immobilization
8. Expression of MMP-8 and MMP-13 at hypertrophic chondrocytes increases after immobilization
9. Immunostaining of type I collagen increases at superficial chondrocytes in the non-contact area and hypertrophic chondrocytes in the transitional and contact areas